INFLUENCE OF WHEAT SEEDLINGS UPON THE HYDROGEN ION CONCENTRATION OF NUTRIENT SOLUTIONS[1]

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Introduction

That the reaction of nutrient culture media bears a very important relation to their biological properties is a fact that is well recognized. Consideration of H ion concentration is of vital importance in connection with plant culture studies, not only because of the profound influence which this factor exerts upon the manner in which plants respond toward certain nutrient elements in the media, but also because of its intimate relation to plant growth in general.

The H ion concentrations of some nutrient solutions commonly used for plant physiological studies undergo rapid and pronounced changes in contact with the roots of growing plants, while the reaction of other nutrient solutions changes only slightly or not at all under similar conditions, owing to the fact that they possess strong buffer properties. The rate, direction, and degree of reaction change are dependent, of course, upon a number of different factors, some of the more important of which are the composition and concentration of the nutrient solutions, and the species, age, and activity of the plants. It is not the purpose of this paper, however, to consider the various factors involved in the relation of the plants to the reaction changes which they may be capable of bringing about in nutrient solutions in which they are grown, but to report briefly an experiment carried out for the purpose of comparing the various nutrient solutions, commonly used for plant cultures, with respect to the initial H ion concentrations of the solutions, and to study the reaction changes induced in them by contact with the roots of young wheat plants.

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Procedure

Spring wheat of the Marquis variety was germinated on a net as described by SHIVE (15). Seedlings carefully selected for uniformity of size and vigor were transferred, when about 5 cm. tall, to SHIVE'S three-salt solution R_5C_2, after having been mounted in the double piece paraffined cork stoppers devised by TOTTINGHAM (18). The stoppers were of the proper size to fit quart fruit jars of colorless glass, which were used for culture vessels. Three seedlings were included in each culture. The cultures thus prepared were conducted during a time period of twenty-five days, with renewal of solutions every three or four days. When the seedlings were approximately thirty days old from the time of germination, cultures were selected in which the plants were about equal with respect to size and vigor in so far as this could be judged from careful observation. The plants of the selected cultures, without being removed from the cork stoppers in which they were mounted, were then taken from the three-salt solutions, the roots were washed by carefully dipping them several times into distilled water, allowed to drain, and were then placed in the nutrient solutions devised by the various authors. The glass jars to which the plants were transferred were like those from which they were removed. Each jar had a capacity of 900 cc.

The formulae of the nutrient solutions devised by the various authors are given in tables I and II in terms of gram-molecules per liter. The solutions made up according to the formulae in table I were corrected to a total osmotic concentration value of approximately 1.75 atmospheres by the method of the freezing point lowering, while those prepared according to the formulae in table II were similarly corrected to an approximate osmotic concentration value of 1.00 atmosphere. No iron was added to any of these solutions, except to those for which this element is mentioned in the formulae. The solutions devised by BIRNER and LUCANUS (1), CRONE (2), and SACHS (13) contained precipitates. In making the cryoscopic tests for the total osmotic concentration values of these solutions the precipitates were allowed to settle, after which samples of the supernatant solutions were drawn off by means of a pipette, the lowering of the freezing points determined, and the corrections made whenever necessary.

TABLE I

FORMULAE OF NUTRIENT SOLUTIONS COMMONLY USED FOR PLANT CULTURES, ALL WITH TOTAL OSMOTIC CONCENTRATION VALUE OF APPROXIMATELY 1.75 ATMOSPHERES AS DETERMINED BY METHOD OF FREEZING POINT LOWERING

Author of solution	Volume-molecular partial concentrations of salts employed											
	KH_2PO_4	$Ca_3(PO_4)_2$	$Ca(H_2PO_4)_2$	$Fe_3(PO_4)_2$	$Ca(NO_3)_2$	KNO_3	$NaNO_3$	$MgSO_4$	K_2SO_4	$CaSO_4$	KCl	$NaCl$
Birner and Lucanus (1)	0.0108	....	....	0.0043	0.0133	....	....	0.0061	....	....	....	....
Crone (2)	....	0.0022	....	0.0019	....	0.0266	....	0.0056	....	0.0049	....	....
Detmer (3)	0.0035	....	....	....	0.0149	....	....	0.0051	....	....	0.0083	....
Hartwell, Wheeler, and Pember (5)	....	....	0.0027	....	0.0136	....	....	0.0077	....	....	0.0075	....
Knop (7)	0.0044	....	....	....	0.0145	0.0059	....	0.0050	....	....	....	....
Pfeffer (12)	0.0041	....	....	....	0.0136	0.0056	....	0.0046	....	....	0.0037	....
Sachs (13)	....	0.0025	....	....	....	0.0155	....	0.0065	....	0.0075	....	0.0076
Schimper (14)	0.0028	....	....	....	0.0018	0.0048	....	0.0040	....	....	....	0.0083
Schreiner, Skinner (16)	....	....	0.0066	....	....	....	0.0278	....	0.0030	....	....	....
Shive, R_5C_2 (15)	0.0180	....	....	....	0.0052	....	....	0.0150	....	....	....	....
Tollens (17)	0.0038	....	....	....	0.0128	0.0052	....	0.0049	....	....	....	0.0054
Tottingham, $T_3R_1C_4$ (18)	0.0108	....	....	....	0.0101	0.0034	....	0.0081	....	....	....	....

The initial H ion concentrations of the nutrient solutions were determined immediately before bringing the plant roots in contact with them. H ion measurements were then repeated for each solution throughout a time period of fifty-two hours, during which the solutions remained in contact with the roots of the growing wheat plants. Nine tests were made of each solution

TABLE II

FORMULAE OF SOME LIVINGSTON-TOTTINGHAM (9) THREE-SALT NUTRIENT SOLUTIONS (TYPES I–VI) AND OF TOTTINGHAM'S (18) SOLUTIONS $T_1R_1C_5$ AND $T_1R_3C_5$ MODIFIED (JONES AND SHIVE 6) BY SUBSTITUTING AMMONIUM SULPHATE FOR POTASSIUM NITRATE IN EQUIVALENT OSMOTIC CONCENTRATIONS; ALL SOLUTIONS HAD TOTAL OSMOTIC CONCENTRATION VALUE OF APPROXIMATELY 1.00 ATMOSPHERE

Type	Number	Volume-molecular partial concentrations									
		KH_2PO_4	$Ca(H_2PO_4)_2$	$Mg(H_2PO_4)_2$	$Ca(NO_3)_2$	$Mg(NO_3)_2$	KNO_3	$MgSO_4$	$CaSO_4$	K_2SO_4	$(NH_4)_2SO_4$
		Livingston-Tottingham three-salt solutions									
I.	R_3S_2	0.0072			0.0048			0.0072			
II.	R_5S_2			0.0019	0.0037					0.0094	
III.	R_4S_1		0.0025				0.0099	0.0074			
IV.	R_4S_1		0.0019			0.0057				0.0076	
V.	R_3S_4			0.0025			0.0074		0.0098		
VI.	R_4S_2	0.0093				0.0047			0.0047		
		Modified Tottingham solutions									
	$T_1R_1C_5$	0.0021			0.0073			0.0071			0.0014
	$T_1R_3C_5$	0.0021			0.0073			0.0024			0.0042

during this period of contact. The small quantities of solutions withdrawn from the culture jars for the purpose of making tests were not replaced, since only about 2 cc. of solution was required for each determination. The H ion concentrations were determined by the colorimetric method, using the double tube color standards described by GILLESPIE (4).

The formulae of the three-salt solutions given in table II were selected from series of the six type-solutions proposed by LIVINGSTON and TOTTINGHAM (9). One solution was selected from each of six series to represent the six different types.[2] The table also

[2] A detailed description of these six type solutions, together with directions for their preparation, may be found in a "plan for cooperative research on the salt requirements of representative agricultural plants prepared for a special committee of the Division of Biology and Agriculture of the National Research Council." Edited by B. E. LIVINGSTON, Baltimore. 1919.

contains the formulae of TOTTINGHAM'S (18) solutions $T_1R_1C_5$ and $T_1R_3C_5$ modified (JONES and SHIVE 6) by substituting ammonium sulphate for the potassium nitrate in equivalent osmotic concentrations. It has previously been shown (6) that in the TOTTINGHAM solutions when thus modified, the direction of the reaction change induced by contact with the roots of young wheat plants is usually the exact opposite of that in the unmodified solutions. It was because of this fact, and also because these modified solutions are capable of producing excellent growth of young wheat plants, that they were included in the experiment.

Discussion

The initial P_H values of the nutrient solutions, the formulae of which are given in table I, and the P_H values determined at intervals during the period of fifty-two hours throughout which the solutions remained in contact with the plant roots, are given in table III. It will be observed from the data of this table that the

TABLE III

P_H VALUES OF NUTRIENT SOLUTIONS DETERMINED AT INTERVALS DURING CONTACT WITH ROOTS OF GROWING WHEAT PLANTS

AUTHOR OF SOLUTION	P_H VALUES									
	INITIAL	Duration of intervals in hours								
		2	4	6	8.5	24.5	27	30.5	33	52
Birner and Lucanus	4.3	4.5	4.6	4.7	4.8	5.0	5.0	5.2	5.2	5.5
Crone	6.6	6.7	6.6	6.5	6.5	6.5	6.5	6.5	6.5	6.6
Detmer	4.7	4.8	4.9	5.0	5.2	5.6	5.6	5.7	5.7	5.9
Hartwell, Wheeler, and Pember	4.0	4.0	4.1	4.5	4.4	4.7	4.7	5.0	5.3	5.7
Knop	4.6	4.7	4.8	5.0	5.1	5.3	5.3	5.4	5.5	5.7
Pfeffer	4.7	4.7	4.9	5.1	5.3	5.5	5.5	5.6	5.7	5.8
Sachs	6.7	6.8	6.6	6.5	6.5	6.5	6.5	6.5	6.5	6.6
Schimper	4.8	4.9	5.1	5.2	5.4	5.7	5.7	5.8	5.9	6.1
Schreiner and Skinner	4.2	4.2	4.4	4.4	4.6	5.4	5.5	5.7	5.9	6.1
Shive, R_5C_2	4.5	4.6	4.6	4.6	4.7	4.8	4.8	5.0	5.1	5.3
Tollens	4.6	4.7	4.9	5.1	5.1	5.5	5.5	5.6	5.9	5.9
Tottingham, $T_3R_1C_4$	4.6	4.7	4.8	4.9	5.0	5.2	5.2	5.3	5.4	5.5

initial P_H values of only two of these solutions are close to the neutral point. CRONE'S (2) solution has an initial P_H value of 6.6, and SACHS'S (13) solution has a corresponding initial value of 6.7.

The initial P_H values of all the other solutions range between 4.0 and 4.8.

The P_H values of CRONE'S and of SACHS'S solutions remained practically unaltered during the entire period of contact with the plant roots. This is probably what might be expected, since the initial P_H values of these solutions lie close to the neutral point, and since the maximum reaction change which the wheat plants are capable of producing in any of the solutions whose formulae appear in table I finally brings the H ion concentrations of these solutions very close to this point, either slightly below or slightly above a P_H value of 7.0, regardless of the initial H ion concentrations of the solutions.

The various solutions exhibit marked differences in the rates of reaction change in contact with the plant roots under similar experimental conditions. Of the solutions with initial P_H values below 5.0, SHIVE'S solution R_5C_2 exhibited the highest resistance to reaction change, while TOTTINGHAM'S solution $T_3R_1C_4$ showed only slightly lower buffer properties as indicated by resistance to reaction change produced by the plants during the fifty-two hour period of contact. On the same basis the solutions of SCHREINER and SKINNER (16), and of HARTWELL, WHEELER, and PEMBER (5) possess relatively low buffer properties. With respect to the solutions here considered, it appears in general that the resistance offered to reaction change resulting from contact with the roots of growing plants is dependent largely upon the volume-molecular proportions of the soluble phosphate salts contained in the solutions. Thus SHIVE'S solution R_5C_2, which contains the highest proportion of dihydrogen potassium phosphate, exhibited the highest buffer properties. This is in entire accord with the observations of MCCALL and HAAG (10), and of MEIER and HALSTEAD (11). In the present experiment, however, there is one striking exception to this general rule as exhibited by the solution of BIRNER and LUCANUS (1), which has a volume-molecular proportion of dihydrogen potassium phosphate equal to that in TOTTINGHAM'S solution $T_3R_1C_4$, and about two and one-half times higher than that in KNOP'S (7) solution or in PFEFFER'S (12) solution, yet these solutions showed a higher resistance to reaction change as influenced by the growing wheat plants than did the solution of BIRNER and LUCANUS. These comparisons, of course, are made upon the assumption that the plants in all the cultures were approximately equal with respect to their ability to cause reaction change.

The P_H values recorded in table IV were determined in the same manner and at the same intervals during the same time period as were those given in table III. The initial H ion concentrations of the LIVINGSTON-TOTTINGHAM (9) solutions containing dihydrogen potassium phosphate are always much lower than

TABLE IV

P_H VALUES OF NUTRIENT SOLUTIONS DETERMINED AT INTERVALS DURING CONTACT WITH ROOTS OF GROWING WHEAT PLANTS

TYPE	NUMBER	P_H VALUES									
		INITIAL	Duration of intervals in hours								
			2	4	6	8.5	24.5	27	30.5	33	52
		Livingston-Tottingham three-salt solutions									
I.	R_3S_2........	4.6	4.7	4.8	5.0	5.1	5.4	5.4	5.5	5.6	5.7
II.	R_5S_2........	3.8	3.8	4.0	4.1	4.3	4.4	4.5	5.0	5.3	5.8
III.	R_4S_1........	3.6	3.6	3.7	3.9	4.0	4.3	4.3	4.6	4.8	5.6
IV.	R_4S_1........	3.7	3.8	3.9	4.0	4.1	4.3	4.3	4.5	4.7	5.6
V.	R_3S_4........	3.6	3.6	3.7	3.8	3.9	4.3	4.3	4.3	4.4	5.4
VI.	R_4S_2........	4.3	4.7	4.7	4.9	5.0	5.2	5.2	5.3	5.4	5.6
		Modified Tottingham solutions									
	$T_1R_1C_5$......	4.8	4.9	4.9	5.0	5.0	4.7	4.6	4.6	4.6	4.2
	$T_1R_3C_5$......	4.8	4.9	4.9	5.0	4.9	4.6	4.4	4.4	4.4	4.2

are those containing the corresponding calcium or magnesium salts. This fact is more certainly brought out by MCCALL and HAAG's (10) table of P_H values determined for six complete series of the LIVINGSTON-TOTTINGHAM type-solutions described in a publication (8) prepared for a special committee of the Division of Biology and Agriculture of the National Research Council.

Mention should be made of the fact that the initial P_H values of the solutions here chosen as representatives of types III, IV, V, and VI, as given in table IV, are not in very close agreement with those determined for the same solutions by MCCALL and HAAG.

The initial P_H values of these solutions in the order given are 3.6, 3.7, 3.6, and 4.3, while McCall and Haag's values are 4.1, 4.1, 4.3, and 4.7 in the same order. Disagreements of this kind are to be expected, of course, because of the different methods employed in determining the P_H values and in the preparation of the solutions, variations in the degree of purity of the salts used, differences in the temperatures of the solutions at the time when the measurements are made, etc. A good example of such discrepancies appears in the P_H values of a series of the Livingston-Tottingham solutions of type I as determined by McCall and Haag and by Meier and Halstead. These authors do not agree upon a single solution of a complete series of twenty-one, the values determined by the latter authors always being considerably but uniformly higher than those determined by the former.

It will be observed from the data of table IV that the maximum reaction changes produced by the plants during the fifty-two hour period of contact with the Livingston-Tottingham solutions with low initial P_H values are always considerably greater than are the corresponding changes in the solutions with higher initial P_H values, since the final P_H values of all these solutions show no very marked differences. Experience with these type-solutions has shown that the maximum reaction changes which young wheat plants are capable of producing in them finally always brings the P_H values of the solutions very close to the neutral point, regardless of the initial P_H values, although the time required to accomplish this may vary considerably with the different solutions, owing to differences in their buffer properties.

As has previously been shown (**6**), the direction of the reaction changes of the modified Tottingham solutions during contact with the roots of the young wheat plants is usually the opposite of that of the Livingston-Tottingham solutions and of the unmodified Tottingham (**18**) solutions under similar conditions. It will be observed, however, that the maximum reaction changes produced in these solutions by the plants during the fifty-two hour period of contact are not very great, the change in the H ion concentration of both solutions here considered being from $P_H=4.8$ to $P_H=4.2$, although the total osmotic concentration value of these solutions is only 1.0 atmosphere.

Experience with twenty representative solutions of TOTTINGHAM'S (18) complete series of eighty-four, modified as here described by substituting ammonium sulphate for the potassium nitrate in equivalent osmotic concentrations, has shown that the P_H values of these solutions are not greatly altered by contact with the roots of young wheat plants between the ages of four and five weeks, the tendency always being toward a slight increase in the H ion concentration of the solutions during growth intervals of three or four days without renewal of the solutions. It is thus easily seen that for certain types of culture studies in which it is desirable to maintain the H ion concentrations of the nutrient media within comparatively narrow variation limits, solutions of this kind possess marked advantages over those in which the H ion concentrations are rapidly decreased by the action of the plants. The two solutions (modified TOTTINGHAM solutions $T_1R_1C_5$ and $T_1R_3C_5$) have the added advantage of high efficiency in the production of young wheat plants when iron in small amounts is supplied to the solutions in an insoluble form such as ferric phosphate. Soluble iron in the form of ferrous sulphate, even in small traces, has been shown (6) to be exceedingly toxic to the plants grown in these solutions.

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